

CORRECTED VERSION

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
31 October 2002 (31.10.2002)

PCT

(10) International Publication Number
WO 2002/085406 A1

- (51) International Patent Classification⁷: A61K 38/26
- (21) International Application Number:
PCT/US2002/013088
- (22) International Filing Date: 24 April 2002 (24.04.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/285,699 24 April 2001 (24.04.2001) US
- (71) Applicant (for all designated States except US):
RESTORAGEN, INC. [US/US]; 4130 NW 37th Street,
Lincoln, NE 68524 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): HOLST, Jens, Juul
[DK/DK]; Ole Olsens Alle 30, DK-2900 Hellerup (DK).
OLSEN, Mette, Zander [DK/DK]; Badstuestraede 8, 2.
t.v., DK-1209 Copenhagen K (DK). HATHAWAY, David,
R. [US/US]; 6201 Oaks Hollow Road, Lincoln, NE 68516
(US).
- (74) Agents: SEASE, Edmund, J. et al.; McKee, Voorhees &
Sease, P.L.C., 801 Grand Avenue, Suite 3200, Des Moines,
IO 50309-2721 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).
- Published:
— with international search report
- (48) Date of publication of this corrected version:
27 May 2004
- (15) Information about Correction:
see PCT Gazette No. 22/2004 of 27 May 2004, Section II
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 2002/085406 A1

(54) Title: METHODS AND COMPOSITIONS FOR TREATING CONDITIONS ASSOCIATED WITH INSULIN RESISTANCE

(57) Abstract: The present invention relates to methods and compositions for treating insulin-associated conditions comprising administering glucagon-like peptide-1 (GLP-1) to subjects suffering therefrom.

-f-

METHODS AND COMPOSITIONS FOR TREATING CONDITIONS
ASSOCIATED WITH INSULIN RESISTANCE

Cross Reference to Related Applications

[0001] The present application claims the benefit of
5 United States Provisional application No. 60/285,699,
filed April 24, 2001.

Field of the Invention

[0002] The present invention relates to endocrinology,
physiology and pharmacology. More particularly, it
10 relates to methods and compositions for treating
conditions associated with insulin resistance.

Background of the Invention

[0003] Insulin resistance is a multi-factorial disease
characterized by the requirement of inappropriately high
15 levels of insulin for maintaining glucose homeostasis.
It is a relatively common disorder that accompanies
obesity, type-2 diabetes, type-2 pre-diabetes, several
disorders including essential hypertension, ventricular
dysfunction with or without congestive symptoms, post-

surgical stress, trauma, polycystic ovary syndrome, burns, gestational diabetes associated with pregnancy, sepsis, myocardial infarction, and stroke, and follows therapy with certain hormones such as glucocorticoids and growth hormone.

5 [0004] Insulin resistance is one of the fundamental components of the insulin resistance syndrome, also known as "syndrome X" or "metabolic syndrome". It is characterized by glucose intolerance, hypertriglyceridemia, reduced levels of high-density lipoprotein cholesterol, hyperinsulinemia and central obesity. Other manifestations of the disease include hypertension, albuminuria, increased serum levels of plasminogen activator inhibitor and increased serum uric acid.

10 [0005] Insulin resistance is an independent risk factor for the development of atherosclerotic vascular disease, which may cause myocardial infarction, stroke, peripheral vascular disease and death. More recently, the insulin resistance and hyperinsulinemia associated with obesity have been linked to cardiac hypertrophy in young individuals.

15 [0006] Current treatment of insulin resistance is limited to that of type-2 diabetes. Metformin and the thiazoladinediones are presently the only drugs available that increase insulin sensitivity. They are effective oral agents for treating the early stages of diabetes, without affecting endogenous secretion of insulin from the pancreas. These drugs may delay or prevent the onset of the later, insulin-dependent phase, as it is widely believed that chronic overproduction of insulin by pancreatic β -cells secondary to profound insulin

20
25
30

resistance leads to depletion of β -cell mass and subsequent insulin-dependent diabetes.

[0007] Because insulin resistance is a risk factor for cardiovascular disease, it often accompanies myocardial infarction and heart failure. In addition, insulin resistance develops or is exacerbated by the rise in neurohormones (e.g. catecholamines, glucocorticoids, TNF- α , etc.) that frequently accompanies these disorders. Metformin and thiazoladinediones are not adequate drugs for treating insulin resistance associated with cardiovascular disease. Metformin is contraindicated in patients with heart disease due to the risk of metabolic acidosis. Moreover, thiazoladinediones are known to cause fluid retention and can precipitate congestive heart failure in patients with left ventricular dysfunction. Hence, this class of drugs is contraindicated in patients with left ventricular dysfunction.

[0008] Insulin resistance also affects skeletal muscle independently of other diseases. It impairs vasodilatation of skeletal muscle blood vessels, reduces transport capacity of glucose into skeletal muscle cells and alters the ratio of fatty acid oxidation versus glucose oxidation as an energy source for oxidative phosphorylation. These effects reduce exercise capacity and contribute independently to ambulatory limitation in obesity and in various cardiovascular diseases, including heart failure.

[0009] There is currently no effective treatment for the insulin resistance-associated conditions or disorders. The available drugs, metformin and the thiazoladinediones, have limited use and are contraindicated in patients with heart disease.

Accordingly, there is a need for new and better compositions and methods for treating insulin-resistance conditions or disorders.

5 Summary of the Invention

[0010] Applicants have solved the above problem by discovering that glucagon-like peptide-1 (GLP-1) is capable of reversing insulin resistance. The present invention relates to methods for treating insulin
10 resistance-associated conditions using GLP-1. In one embodiment, the insulin resistance-associated condition is selected from the group consisting of type-2 pre-diabetes, atherosclerotic cardiovascular disease (ASCVD), drug-induced insulin resistance, congestive heart
15 failure, diminished exercise capacity of skeletal muscle, and left ventricular dysfunction with cardiac metabolic myopathy or diminished exercise capacity of skeletal muscle, with the proviso that said congestive heart failure is not associated with toxic hypervolemia.

20 [0011] In another embodiment, the GLP-1 is selected from the group consisting of GLP-1(7-36)NH₂, GLP-1(9-36)NH₂, GLP-4(7-37), and exendin-4.

[0012] In yet another embodiment, the GLP-1 is administered for a period of time of at least 6, 8, 10,
25 12 or 14 weeks. In another embodiment, the subject is a human.

Brief Description of the Drawings

FIG. 1. Plasma glucose concentrations during 8-h
30 profiles. Fig. 1A represents plasma glucose levels in the GLP-1 and saline group before infusion of GLP-1 and

saline respectively was started. Fig. 1B represents plasma glucose levels of patients infused with saline at week 0, week 1 and week 6.

5 FIG. 2. Plasma glucose levels in GLP-1 treated patients at week 0, week 1 and week 6.

10 FIG. 3. Measurement of the insulin levels at weeks 0 and 6 for saline and GLP-1 treated patients. Insulin sensitivity was measured using the hyperinsulinemic euglycemic clamp, before infusion was started (week 0) and after 6 weeks of infusion (week 6).

15 FIG. 4. Measurement plasma glucose levels during the clamp in Fig. 3 at weeks 0 and 6 for saline and GLP-1 treated patients.

20 FIG. 5. Measurement of insulin sensitivity (mg glucose/kg fat free mass/min) at weeks 0 and 6 for saline and GLP-1 treated patients during the clamp in Fig. 3.

25 FIG. 6. Measurement of β -cell function (C-peptide levels from the hyperglycemic clamp). The upper curves represent C-peptide levels in patients receiving saline. The lower curves represent C-peptide levels in GLP-1 treated patients. Incremental area under the curve (AUC) 0-90, first phase responses (incremental AUC 0-10), second phase responses (incremental AUC 10-45) and maximal secretory capacity (incremental peak levels after
30 glucose plus arginine) all significantly increased. P values are shown in the result section. The small inserted figure represents C-peptide levels from 0-20 minutes.

FIG. 7. The effect of dilated cardiomyopathy on norepinephrine, insulin, free fatty acid and glucose levels in a dog model.

5

FIG. 8. The effect of GLP-1 on myocardial free fatty acid/glucose uptake ratio in dilated cardiomyopathy.

FIG. 9. The effect of GLP-1 on cardiac function in dilated cardiomyopathy. FIG. 9A represents the effect of GLP-1 on stroke volume. FIG. 9B represents the effect of GLP-1 on left ventricular systolic pressure. FIG. 9C represents the effect of GLP-1 on left ventricular contractility. FIG. 9D represents the effect of GLP-1 on heart rate.

10
15

Detailed Description of the Invention

[0013] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. All publications, patents and other references mentioned herein are incorporated by reference.

[0014] Although methods and materials similar or equivalent to those described herein can be used in the practice of testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are for illustrative purposes only, and are not intended to be limiting. Other features and advantages of the invention will be

20
25
30

apparent from the detailed description and from the claims.

[0015] Throughout this specification, the word "comprise" or variations such as "comprises" or
5 "comprising" will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers.

[0016] In order to further define the invention, the following terms and definitions are herein provided.

10 [0017] The terms "GLP-1," "GLP-1 molecule," "glucagon-like peptide-1," or "glucagon-like peptide-1 molecule" according to this invention include GLP-1 as well as biologically active variants, analogs, mimetics, agonists, and derivatives thereof. "Biologically active"
15 in this context means having the biological activity of GLP-1(7-36) amide (GLP-1(7-36)NH₂), but it is understood that the activity of the variant, analog, mimetic, agonist, or derivative thereof can be either less potent or more potent than native GLP-1(7-36)amide. The
20 agonists of GLP-1, as well as GLP-1 mimetics that function as agonists, include, e.g., chemical compounds specifically designed to activate the GLP-1 receptor.

[0018] The term "insulin resistance" as used herein, refers to a subnormal biological response to a given
25 concentration of insulin.

[0019] The term "insulin resistance-associated condition", "condition associated with insulin resistance", "insulin resistance-associated disorder" or
30 "disorder associated with the insulin resistance" as used herein refers to any disease, disorder or condition whereby insulin resistance is a comorbid condition that contributes, exacerbates or interacts synergistically with symptoms, signs, prognosis or clinical outcome.

Insulin resistance-associated condition includes but is not limited to type-2 pre-diabetes, ASCD, drug-induced insulin resistance, congestive heart failure which is not associated with toxic hypervolemia, myocardial
5 infarction, stroke, left ventricular dysfunction with cardiac metabolic myopathy and diminished exercise capacity of skeletal muscle.

[0020] The term "insulin regulating" as used herein, refers to an ability to control the release of insulin
10 into the circulation, in relation to blood glucose and fatty acid levels.

[0021] The term "pharmaceutically acceptable carrier or adjuvant" as used herein, refers to a non-toxic carrier or adjuvant that may be administered to a patient
15 together with a compound of the invention, and which does not destroy the pharmacological activity thereof.

[0022] The terms "therapeutically or pharmaceutically effective" or "therapeutically or pharmaceutically effective amount" refers to an amount of the compound of
20 this invention required to reduce or lessen the severity of insulin resistance for some period of time. A therapeutically or pharmaceutically effective amount also means the amount required to improve the clinical symptoms.

25 [0023] The present invention relates to methods for treating conditions associated with insulin resistance in a subject. The methods include administering to a subject a therapeutically effective amount of GLP-1. The methods of this invention further relate to decreasing
30 insulin resistance using GLP-1.

Glucagon-Like Peptide-1 (GLP-1)

[0024] GLP-1 plays a key role in the regulation of plasma glucose homeostasis. It is involved in regulating insulin secretion in relation to blood glucose and/or lipid levels, and inhibiting glucagon release by the pancreas, inhibiting gastric acid secretion and motility, and suppressing appetite and food intake. GLP-1 is a member of the incretin group of secretagogue hormones that are released from the intestinal enteroendocrine cells in response to the ingestion of food. GLP-1 binds to the GLP-1 receptors, some of which are expressed on the β -cells of the pancreas. Binding of GLP-1 to its receptor triggers an intracellular signaling pathway that results in controlling insulin secretion with concomitant inhibition of glucagon production. This in turn leads to uptake of glucose in muscle and fat cells and the inhibition of hepatic glucose production, all of which lowers blood glucose levels. Although the role of GLP-1 in maintaining plasma glucose concentration is well established, prior to this invention, it was not known that GLP-1 is also capable of increasing insulin sensitivity.

[0025] GLP-1 is an intestinally produced peptide hormone. Under physiological conditions, GLP-1 is secreted in response to a meal (Kreymann, B. et al., *Lancet* 2: 1300-1304 (1987) and has an important role in regulating post-prandial glucose levels (Edwards, C.M. et al., *Diabetes* 48: 86-93 (1999)). GLP-1 secretion is reduced in type-2 diabetic patients (Lugari, R. et al., *Horm. Metab. Res.* 32: 424-428 (2000)). Exogenous GLP-1 stimulates insulin secretion, inhibits glucagon secretion and reduces plasma glucose in type-2 diabetic patients (Gutniak, M. et al., *N. Engl. J. Med.* 326: 1316-1322

(1992); Nauck, M.A. et al., *Diabetologia* 36: 741-744 (1993)).

[0026] Furthermore, GLP-1 reduces appetite in both obese (Naslund, E. et al., *Int. J. Obes. Relat. Metab. Disord.* 23: 304-311 (1999)) and type-2 diabetic subjects (Toft-Nielsen, M.B. et al., *Diabetes Care* 22: 1137-1143 (1999)). GLP-1 has been shown to reverse age-dependent decline in β -cell function in rats (Wang, Y. et al., *J. Clin. Invest.* 99: 2883-2889 (1997)). It has also
10 recently been shown to stimulate β -cell proliferation and neogenesis in rats (Perfetti, R. et al., *Endocrinology* 141: 4600-4605 (2000); Xu, G. et al., *Diabetes* 48: 2270-2276 (1999)).

[0027] As used herein, a "GLP-1 molecule" includes the
15 following compounds. Mammalian GLP peptides and glucagon are encoded by the same gene. In the ileum, the precursor is processed into two major classes of GLP peptide hormones, namely GLP-1 and GLP-2. GLP-1(1-37) has the sequence: His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-
20 Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQ ID NO: 1). GLP-1(1-37) is amidated post-translationally to yield GLP-1(1-36)NH₂, which has the sequence: His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-
25 Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg(NH₂) (SEQ ID NO: 2), or is enzymatically processed to yield GLP-1(7-37), which has the sequence: His-Ala-Glu-Gly-Thr-Phe-Thr-
30 Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly-(SEQ ID NO 3). GLP-1(7-37) can also be amidated to yield GLP-1(7-36)amide, which has the sequence: His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-

Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg(NH₂) (SEQ ID NO: 4). Likewise, GLP-1(1-36)amide can be processed to GLP-1(7-36)amide.

[0028] Intestinal L cells secrete GLP-1(7-37) (SEQ ID NO: 3) and GLP-1(7-36)NH₂ (SEQ ID NO: 4) in a ratio of 1:5. These truncated forms of GLP-1 have short half-lives *in vivo* (less than 10 minutes), and are inactivated by an aminodipeptidase (DPP IV) to yield GLP-1(9-37), which has the sequence: Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQ ID NO: 5), and GLP-1(9-36)amide, which has the sequence: Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg(NH₂) (SEQ ID NO: 6), respectively. It has been speculated that the peptides GLP-1(9-37) and GLP-1(9-36)amide might affect hepatic glucose production, but apparently they do not stimulate production or release of insulin from the pancreas.

[0029] As used in this specification, the term "GLP-1 molecule" includes GLP-1(1-37), GLP-1(1-36)NH₂, GLP-1(7-37), and GLP-1(7-36)NH₂ ("GLP-1(7-36)amide") (collectively referred to as "GLP-1 peptides"). The present invention includes the use of recombinant human GLP-1 peptides and GLP-1 peptides derived from other species, whether recombinant or synthetic.

[0030] "GLP-1 molecule" further denotes biologically active variants, analogs, and derivatives of GLP-1 peptides. "Biologically active," in this context, means having GLP-1(7-36) biological activity, but it is understood that the variant, analog, or derivative can be either less or more potent than GLP-1(7-36)amide, a native, biologically active form of GLP-1. See Göke & Byrne, *Diabetic Medicine*. 13: 854 (1996). GLP-1

molecules of the present invention also include polynucleotides that express agonists of GLP-1 (*i.e.*, activators of the GLP-1 receptor molecule and its secondary messenger activity found on, *inter alia*, insulin-producing β -cells). GLP-1 mimetics that also are agonists of GLP-1 receptors include, for example, chemical compounds designed or anticipated to activate the GLP-1 receptor.

[0031] Included in GLP-1 molecules are any molecules, whether they be peptides, peptide mimetics, or other molecules, that bind to or activate a GLP-1 receptor, such as the GLP-1(7-36)amide receptor, and its second messenger cascade. GLP-1 molecules include species having insulin regulating activity and that are agonists of (*i.e.*, activate), the GLP-1 receptor molecule and its second messenger activity on, *inter alia*, insulin producing β -cells.

[0032] "GLP-1 molecules" also include peptides that are encoded by polynucleotides that express biologically active GLP-1 variants, as defined herein. Also included in the present invention are GLP-1 molecules that are peptides containing one or more amino acid substitutions, additions or deletions, compared with GLP-1(7-36)amide. In one embodiment, the number of substitutions, deletions, or additions is 30 amino acids or less, 25 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less or any integer in between these amounts. In one aspect of the invention, the substitutions include one or more conservative substitutions. A "conservative" substitution denotes the replacement of an amino acid residue by another, biologically active similar residue. Examples of conservative substitutions include the

substitution of one hydrophobic residue, such as isoleucine, valine, leucine, or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic
 5 for aspartic acids, or glutamine for asparagine, and the like. The following table lists illustrative, but non-limiting, conservative amino acid substitutions.

<u>ORIGINAL RESIDUE</u>	<u>EXEMPLARY SUBSTITUTIONS</u>
ALA	SER, THR
ARG	LYS
ASN	HIS, SER
ASP	GLU, ASN
CYS	SER
GLN	ASN, HIS
GLU	ASP, GLU
GLY	ALA, SER
HIS	ASN, GLN
ILE	LEU, VAL, THR
LEU	ILE, VAL
LYS	ARG, GLN, GLU, THR
MET	LEU, ILE, VAL
PHE	LEU, TYR
SER	THR, ALA, ASN
THR	SER, ALA
TRP	ARG, SER
TYR	PHE
VAL	ILE, LEU, ALA
PRO	ALA

[0033] It is further understood that GLP-1 peptide
 10 variants include the above described peptides which have been chemically derivatized or altered, for example,

peptides with non-natural amino acid residues (e.g., taurine, β - and γ -amino acid residues and D-amino acid residues), C-terminal functional group modifications, such as amides, esters, and C-terminal ketone
5 modifications and N-terminal functional group modifications, such as acylated amines, Schiff bases, or cyclization, as found—for example—in the amino acid pyroglutamic acid.

[0034] Also included in the present invention are
10 peptide sequences having greater than 50% sequence identity, and preferably greater than 90% sequence identity to (1) SEQ ID NOS: 1, 2, 3, 4; and (2) to truncated sequences thereof. As used herein, sequence identity refers to a comparison made between two
15 molecules using standard algorithms well known in the art. The preferred algorithm for calculating sequence identity for the present invention is the Smith-Waterman algorithm, where SEQ ID NO:1 [i.e., GLP-1(1-37)] is used as the reference sequence to define the percentage
20 identity of homologs over its length. The choice of parameter values for matches, mismatches, and insertions or deletions is arbitrary, although some parameter values have been found to yield more biologically realistic results than others. One preferred set of parameter
25 values for the Smith-Waterman algorithm is set forth in the "maximum similarity segments" approach, which uses values of 1 for a matched residue and $-\frac{1}{2}$ for a mismatched residue (a residue being either a single nucleotide or single amino acid). Waterman, *Bull. Math. Biol.* 46: 473
30 (1984). Insertions and deletions (indels), x , are weighted as $x_k = 1 + \frac{1}{2}k$, where k is the number of residues in a given insert or deletion. *Id.*

[0035] For instance, a sequence that is identical to the 37-amino acid residue sequence of SEQ ID NO: 1, except for 18 amino acid substitutions and an insertion of 3 amino acids, would have a percent identity given by:

$$\begin{aligned} & [(1 \times 37 \text{ matches}) - (\frac{1}{2} \times 18 \text{ mismatches}) \\ & - (1 + \frac{3}{3} \text{ indels})] / 37 = 78\% \text{ 'identity'} \end{aligned}$$

Also included in "GLP-1 molecules" of the present invention are six peptides in Gila monster venoms that are homologous to GLP-1. Their sequences are compared to the sequence of GLP-1 in Table 1.

TABLE 1

15	a.	H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R (NH ₂)
	b.	H S D G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S (NH ₂)
	c.	D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S (NH ₂)
	d.	H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S (NH ₂)
	e.	H S D A T F T A E Y S K L L A K L A L Q K Y L E S I L G S S T S P R P P S
	f.	H S D A T F T A E Y S K L L A K L A L Q K Y L E S I L G S S T S P R P P S
20	g.	H S D A I F T E E Y S K L L A K L A L Q K Y L A S I L G S R T S P P P (NH ₂)
	h.	H S D A I F T Q Q Y S K L L A K L A L Q K Y L A S I L G S R T S P P P (NH ₂)

- a = GLP-1(7-36)amide (SEQ. ID NO:4)
 b = exendin 3 (SEQ. ID NO:7).
 25 c = exendin 4 (9-39(NH₂)) (SEQ. ID NO:8).
 d = exendin 4 (SEQ. ID NO:9).
 e = helospectin I (SEQ. ID NO:10).
 f = helospectin II (SEQ. ID NO:11).
 g = helodermin (SEQ. ID NO:12).
 30 h = Q⁸, Q⁹ helodermin (SEQ. ID NO:13).

[0036] Peptides (a, b, d, e, f, and g) are homologous at positions 1, 7, 11 and 18. GLP-1 and exendins are further homologous at positions, 4, 5, 6, 8, 9, 15, 22, 35 23, 25, 26 and 29. In position 2, A, S, and G are structurally similar. In position 3, residues D and E

(Asp and Glu) are structurally similar. In positions 22 and 23, F (Phe) and I (Ile) are structurally similar to Y (Tyr) and L (Leu), respectively. Likewise, in position 26, L and I are structurally similar.

5 [0037] Thus, of the 30 residues of GLP-1, exendins 3 and 4 are identical in 15 positions and equivalent in 5 additional positions. The only positions where major structural changes are evident are at residues 16, 17, 10 19, 21, 24, 27, 28 and 30. Exendins also have 9 extra residues at the C-terminus.

[0038] Agonists of glucagon-like peptide that exhibit activity through the GLP-1(7-36)amide receptor have been described. See EP 0708179 A2; Hjorth et al., *J. Biol. Chem.* 269: 30121 (1994); Siegel et al., *Amer. Diabetes Assoc. 57th Scientific Session*, Boston (1997); Hareter et al., *Amer. Diabetes Assoc. 57th Scientific Session*, Boston (1997); Adelhorst et al., *J. Biol. Chem.* 269: 6275 (1994); Deacon et al., 16th International Diabetes Federation Congress Abstracts, *Diabetologia Supplement* 20 (1997); Irwin et al., *Proc. Natl. Acad. Sci. USA* 94: 7915 (1997); Mojsov, *Int. J. Peptide Protein Res.* 40: 333 (1992); Göke & Byrne, *Diabetic Medicine* 13: 854 (1996). Recent publications disclose Black Widow GLP-1 and Ser² GLP-1. See Holz & Hakner, *Comp. Biochem. Physiol.*, Part 25 B 121: 177 (1998) and Ritzel et al., *J. Endocrinol* 159: 93 (1998).

[0039] GLP-1 receptors are cell-surface proteins found, for example, on insulin-producing pancreatic β -cells; GLP-1(7-36) receptors and variants thereof have 30 been characterised in the art. Methods of determining whether a chemical or peptide binds to or activates a GLP-1 receptor are known to the skilled artisan.

[0040] The biological activity of a GLP-1 molecule can be determined by *in vitro* and *in vivo* animal models and human studies, as is well known to the skilled artisan. GLP-1 biological activity can be determined by standard methods, in general, by receptor-binding activity screening procedures, which involve providing appropriate cells that express the GLP-1 receptor on their surface, for example, insulinoma cell lines such as RINmSF cells or INS-1 cells. See Mojsov, *Int. J. Peptide Protein Res.* 40: 333 (1992) and EP 0708179. Cells that are engineered to express a GLP-1 receptor also can be used. In addition to measuring specific binding of tracer to membrane using radioimmunoassay methods, cAMP activity or glucose dependent insulin production can also be measured. In one method, a polynucleotide encoding the GLP-1 receptor is employed to transfect cells so that they express the GLP-1 receptor protein. Thus, for example, these methods may be employed for screening for a receptor agonist by contacting such cells with compounds to be screened and determining whether such compounds generate a signal (*i.e.*, activate the receptor). Other screening techniques include the use of cells that express the GLP-1 receptor, for example, transfected CHO cells, in a system to measure extracellular pH or ionic changes caused by receptor activation. For example, potential agonists may be contacted with a cell that expresses the GLP-1 receptor and a second messenger response (*e.g.*, signal transduction or ionic or pH changes), may be measured to determine whether the potential agonist is effective.

[0041] Polyclonal and monoclonal antibodies can be utilized to detect, purify, and identify GLP-1-like peptides for use in the methods described herein.

Antibodies such as ABGA1178 detect intact GLP-1(1-37) or N-terminally-truncated GLP-1(7-37) or GLP-1(7-36)amide. Other antibodies detect the end of the C-terminus of the precursor molecule, a procedure that allows one-by subtraction-to calculate the amount of biologically active, truncated peptide (*i.e.*, GLP-1(7-37)amide). Orskov *et al.*, *Diabetes* 42; 658 (1993); Orskov *et al.*, *J. Clin. Invest.* 1991, 87; 415 (1991).

5
10
15
[0042] The GLP-1 molecules of the invention that are peptides that can be made by solid-state chemical peptide synthesis. Such peptides can also be made by conventional recombinant techniques using standard procedures described in, for example, Sambrook & Maniatis. "Recombinant," as used herein, means that a gene is derived from a recombinant (*e.g.*, microbial or mammalian) expression system that has been genetically modified to contain a polynucleotide encoding a GLP-1 molecule as described herein.

20
25
[0043] The GLP-1 molecule peptides of the present invention may be a naturally purified product, or a product of synthetic chemical procedures, or produced by recombinant techniques from prokaryotic or eukaryotic hosts (for example, by bacteria, yeast, higher plant, insect, or mammalian cells in culture or *in vivo*). Depending on the host employed in a recombinant production procedure, the polypeptides of the present invention are generally non-glycosylated, but may be glycosylated.

30
[0044] The GLP-1 like peptides can be recovered and purified from recombinant cell cultures by methods including, but not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography,

hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography, and lectin chromatography. High-performance liquid chromatography (HPLC) can be employed for purification steps.

5 [0045] Particularly preferred GLP-1 molecules of the invention are GLP-1(7-36)amide, GLP-1(7-37), and exendin-4.

Uses for GLP-1

10 [0046] The methods and compositions of this invention may be used to treat conditions associated with insulin resistance. Insulin resistance is associated with several conditions including type-2 pre-diabetes, atherosclerotic cardiovascular disease (ASCVD), drug-
15 induced insulin resistance, congestive heart failure which is not associated with toxic hypervolemia, diminished exercise capacity of skeletal muscle, and left ventricular dysfunction with at least one of cardiac metabolic myopathy or reduced exercise capacity of
20 skeletal muscle. Therefore, the present invention provides methods of treating conditions associated with insulin resistance comprising the step of administering GLP-1.

[0047] Insulin resistance may be due to any one or
25 more events including abnormal prereceptor (e.g., abnormal ligand or competition), receptor (e.g., abnormal structure, affinity of ligand to receptor, or number of receptors), or postreceptor (e.g., abnormal signaling) events. Insulin resistance may be determined by a number
30 of methods known in the art. For example, the euglycemic hyperinsulinemic clamp technique may be used to diagnose insulin resistance (Rao, G., *Am. Fam. Physician* (2001) 63: 1159-63). This technique involves intravenous

administration of an insulin dose while simultaneously maintaining glucose at a pre-set level within the normal range by also administering glucose. At equilibrium, the amount of glucose uptake by a particular tissue in the presence of a certain dose of insulin can be calculated. Other methods used to detect insulin resistance include the insulin suppression test, intravenous glucose tolerance test, and constant infusion of glucose with model assessment (Rao, G., *supra*).

10 [0048] Insulin resistance uniformly accompanies type-2 pre-diabetes. However, only a small fraction (i.e., 10-20%) of people with insulin resistance actually develop frank type-2 diabetes mellitus. Therefore, insulin resistance is a distinct disorder. Accordingly, in one embodiment, the invention provides a method for treating insulin resistance associated with type-2 pre-diabetes comprising the step of administering GLP-1.

15 [0049] Insulin resistance has an important role as an independent risk factor for the development of atherosclerotic cardiovascular diseases (ASCD). For example, hyperinsulinemia is an independent risk factor for coronary artery disease. In addition, high levels of circulating insulin are associated with hypertension, which may result from higher sympathetic nerve activity. Accordingly, another embodiment of the invention provides a method for treating ASCD comprising the step of administering GLP-1.

25 [0050] Moreover, insulin resistance develops as a consequence of therapy with certain hormones, including but not limited to, glucocorticoids or growth hormone. Accordingly, in one embodiment, the invention provides a method for treating drug-induced insulin resistance comprising the step of administering GLP-1. In a

30

preferred embodiment, the drug is selected from the group consisting of glucocorticoid and growth hormone.

[0051] Insulin resistance also develops as an associated disease that contributes to overall morbidity and mortality in other diseases, such as congestive heart failure which is not associated with toxic hypervolemia, left ventricular dysfunction secondary to myocardial injury and diminished skeletal muscle exercise capacity. Neurohormones such as catecholamines, glucagon, cortisol, growth hormone and cytokines such as TNF- α are all known to induce insulin resistance alone or in combination. In some cases, these neurohormones initiate insulin resistance in the aforementioned diseases, as associated stress causes many of the neurohormones to be secreted into the blood. In other cases, the neurohormones may simply exaggerate the underlying resistance.

[0052] Insulin resistance alters the metabolism and function of tissues that use both fatty acids and glucose as substrates for oxidative phosphorylation (e.g., oxygen-dependent ATP synthesis). This is most evident in cardiac and skeletal muscle. For example, in diseases associated with severely depressed left ventricular dysfunction and reduced peripheral blood flow, insulin resistance develops subsequent to the rise in serum neurohormones. The resistance is tantamount to insulin deficiency. Normally, insulin drives the oxidation of glucose via enhanced transport of glucose into muscle and via activation of the enzyme, pyruvate dehydrogenase. Insulin promotes the oxidation of glucose and reduces the oxidation of fatty acids. With insulin resistance the effect is lost and fatty acid oxidation is preferred. Fatty acids are oxidized to produce ATP at lower overall efficiency. That is, more oxygen is required to produce

each ATP molecule compared to stoichiometric amounts of glucose.

[0053] When cardiac output is compromised by left ventricular dysfunction (e.g., that may occur subsequent to myocardial ischemia, infarction or cardiomyopathy) the reduced efficiency of fatty acid oxidation may further compromise heart function. In fact, in chronic states of depressed cardiac output, other changes in cardiac metabolism may amplify the inefficiency problem. For example, the accumulation of long chain acyl-CoA molecules and inhibition of the transport of fatty acids in the mitochondria may uncouple oxidative phosphorylation, causing more oxygen consumption at even lower rates of ATP synthesis. Such patients acquire a new disease, namely, a reversible metabolic myopathy. This disease is characterized by a disproportionate preference for fatty acid over glucose for oxidative ATP synthesis, a reduced ATP/O₂ ratio and depressed indices of cardiac performance. Accordingly, in another embodiment, the invention provides a method for treating insulin resistance associated with a condition selected from the group consisting of congestive heart failure and left ventricular dysfunction with cardiac metabolic myopathy or diminished exercise capacity of skeletal muscle, comprising the step of administering GLP-1; with the proviso that said congestive heart failure is not associated with toxic hypervolemia.

[0054] Insulin resistance is also associated with impaired skeletal muscle vasodilatation during exercise in the presence or absence of obesity, or left ventricular dysfunction. This impaired skeletal muscle vasodilatation diminishes exercise capacity. Moreover, glucose uptake and utilization by skeletal muscle are

likewise impaired in patients with insulin resistance. Accordingly, in another embodiment, the invention provides a method for treating insulin resistance associated with diminished exercise capacity of skeletal muscle, in the presence or absence of obesity, left ventricular dysfunction or congestive heart failure comprising the step of administering GLP-1; with the proviso that said congestive heart failure is not associated with toxic hypervolemia. In one embodiment, the insulin resistance associated with diminished exercise capacity is in the presence of obesity, left ventricular dysfunction or congestive heart failure, which is not associated with toxic hypervolemia. In another embodiment, the insulin resistance associated with diminished exercise capacity of skeletal muscle is in the absence of obesity, left ventricular dysfunction or congestive heart failure, which is not associated with toxic hypervolemia.

20 Pharmaceutical Compositions

[0055] The GLP-1 molecules may be formulated into pharmaceutical compositions for administration to subjects, including humans. These pharmaceutical compositions, preferably include an amount of GLP-1 effective to treat insulin resistance and a pharmaceutically acceptable carrier.

[0056] Pharmaceutically acceptable carriers useful in these pharmaceutical compositions include, e.g., ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such

as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0057] The compositions of the present invention may be administered parenterally, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

Preferably, the compositions are administered by infusion or subcutaneous injection of a slow-release formulation.

[0058] Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation

of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain
5 alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other
10 commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

15 [0059] Parenteral formulations may be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions may be administered once a day or on an "as needed" basis.

[0060] The pharmaceutical compositions of this
20 invention may be orally administered in any orally acceptable dosage form including, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium
25 stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If
30 desired, certain sweetening, flavoring or coloring agents may also be added.

[0061] Alternatively, the pharmaceutical compositions of this invention may be administered in the form of

suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[0062] The pharmaceutical compositions of this invention may also be administered topically. Topical application can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[0063] For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0064] For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic

uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

[0065] The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0066] The amount of GLP-1 molecule that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. The compositions can be formulated so that a dosage of between 0.1-1000 pmoles/kg body weight/minute (when administered by infusion) of GLP-1 molecule is administered to a patient receiving these compositions. In some embodiments of the invention, the dosage is 0.1-10 pmoles /kg body weight/minute when administered by infusion). In a preferred embodiment, the dosage is 0.5 - 2.0 pmoles/kg/min/when administered by intravenous infusion. The composition may be administered as a single dose, multiple doses or over an established period of time in an infusion.

[0067] In some embodiments, the pharmaceutical composition may be administered to a subject for a period of time including, but not limited to, at least 6, 8, 10, 12 or 14 weeks. Other long-term treatments with a pharmaceutical composition of this invention may be suitable.

[0068] In a preferred embodiment, GLP-1 is administered to patients with a confirmed condition associated with insulin resistance. In another preferred embodiment, GLP-1 is administered by injection at least
5 once a day or by continuous infusion via pump. In yet another preferred embodiment, GLP-1 is formulated for administration from a subcutaneous depot over a period of days to weeks, oral administration or by intermittent inhalation.

10 [0069] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular GLP-1 molecule, the patient's age, body weight, general health, gender, and diet, and the time of administration, rate of excretion, drug
15 combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within ordinary skill in the art. The amount of GLP-1 molecule will also depend on the individual patient to be treated, the route of
20 administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amounts of GLP-1 molecules can be determined by pharmacological and pharmacokinetic principles well known in the art.

25 [0070] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

Examples1. Effect of GLP-1 in HumansA. Subjects and Protocol

[0071] In a study that was blinded to the subjects,
 5 all patients participating had been diagnosed with type-2
 diabetes after the age of 40. All patients had normal
 hemoglobin, creatinine and liver parameters. Three weeks
 prior to the study, oral antidiabetic medication was
 discontinued. In order to make the study as blinded as
 10 possible to the patients, home blood glucose measurements
 were not allowed.

[0072] Twenty patients were randomly allocated into
 two groups; one group received GLP-1 while the other
 received saline. There were no significant differences
 15 in age, body mass index (BMI), HbA_{1c} or fasting plasma
 glucose (FPG) levels between the two groups. Clinical
 characteristics of the patients prior to infusion of GLP-
 1 or saline are shown in Table 2. One patient in the
 saline group whose veins were too small for possible
 20 blood sampling was excluded from the study.

TABLE 2

	Female /Male	Mean (Range of) Age in Years	BMI (kg/m ²)	FPG (mmol/l)	HbA _{1c} (%)
GLP-1	4/6	55 (46-61)	34.9±1.8	14.4±1.0	9.2±0.6
Saline	3/6	54 (47-64)	31.9±1.2	13.2±1.0	8.9±0.4

[0073] All patients underwent a hyperglycemic clamp, a
 25 dexascan, a hyperinsulinemic euglycemic clamp, and a
 measurement of gastric emptying. 8-hour (8-h) profiles

of plasma glucose, insulin, C-peptide, glucagon and free fatty acids were obtained immediately before the infusions were started (week 0) to define baseline values. The patients were then equipped with a portable
5 insulin pump, MINIMED 506 (Minimed, Sweden) for continuous subcutaneous infusion of saline or GLP-1. Recombinant GLP-1 (7-36)amide (BioNebraska, Lincoln, Nebraska) was supplied as a 1 mg/ml liquid sterile formulation, with a peptide purity of >99% as determined
10 by reversed-phase HPLC. The infusion rate was 4.8 pmol/kg/min. The portable insulin pump was to be used continuously (day and night) for the next 6 weeks. Patients receiving GLP-1 were instructed about filling up their pumps every 24 hours. Patients receiving saline
15 had their pumps filled at the hospital once a week. [0074] After one week of infusions, the patients underwent a hyperglycemic clamp, measurement of gastric emptying and 8-h profiles were obtained. After 6 weeks of infusion (week 6), all procedures from week 0 were
20 repeated. GLP-1 was measured twice daily on examination days and once weekly between week 1 and week 6 in order to monitor pump function and patient compliance. HbA_{1c} was measured at week 0 and 6, fructosamine at week 0, 3 and 6. Between week 1 and 6, the patients were monitored
25 for control of blood glucose, body weight, blood pressure and pulse.

B. Hyperglycemic Clamp

[0075] The clamp level was 30 mmol/l for 90 min and 5g
30 L-arginine hydrochloride in saline was infused after 45 minutes. See Ward, W.K. et al. *J. Clin. Invest.* 74: 1318-1328 (1984), incorporated herein by reference.

C. Dual-Energy X-ray Absorptiometry

[0076] Body composition and weight was estimated by a DXA scanner, Norland XR 36 (Norland Instruments, Fort Atkinson, WI, USA). See Gotfredsen, A. et al. *J. Appl. Physiol.* 82: 1200-1209 (1997), incorporated herein by reference.

D. Hyperinsulinemic Euglycemic Clamp

[0077] Insulin was administered as a primed infusion at a rate of 40 mU/m²/minute in order to raise the plasma insulin level by 100 µU/ml. See DeFronzo, R.A. et al. *Am. J. Physiol.* 237: E214-E223 (1979), incorporated herein by reference. At euglycemia (5.0 mmol/l), the clamp was maintained for 120 minutes.

15

E. Measurement of gastric emptying and 8-h profile of substrates and hormones

[0078] Gastric emptying was measured using the gamma camera technique. At 9 AM following an overnight fast, the patients ingested a 1.6-MJ meal within 10 minutes. As a solid marker, 20 MBq of 99mTc-stannous colloid was added to the meal. Anterior and posterior images were obtained (MaxiCamera 400, General Electric, Milwaukee, WI), with each acquisition lasting 2 minutes and being repeated at 15-minute intervals for 3 hour. Four hours after breakfast, the patients received a fixed meal for lunch. Sensations of hunger, satiety, fullness, prospective food consumption, nausea and well-being were rated on 100-mm visual analogue scales before and after the two meals as well as 2 hours post-prandially (Flint, A. et al. *Int. J. Obes. Relat. Metab. Disord.* 24: 38-48 (2000)). Plasma glucose, insulin, C-peptide, glucagon

and free fatty acid (FFA) levels were determined every half hour.

F. Analytic Procedures

5 [0079] Plasma glucose concentrations were analyzed using a Beckman Analyzer (Beckman Instrument, Fullerton, CA). The glucagon assay (Orskov, C. et al. *J. Clin. Invest.* 87: 415-423 (1991)) is directed against the COOH-terminus of the glucagon molecule (antibody code no. 10 4305). Total GLP-1 was measured by radioimmunoassay employing antiserum code no. 89390 which is highly specific for the COOH-terminus of GLP-1 and therefore measures the sum of GLP-1(7-36) amide and its metabolite GLP-1 (9-36)amide (Orskov, C., et al. *Diabetes* 43: 535-539 15 (1994)). For glucagon and GLP-1 analysis, plasma was extracted with ethanol (final concentration 70%vol/vol) before analysis. Detection limits and intra-assay coefficients of the assays used were 1 pmol/l and <6% for glucagon, 1 pmol/l and <6% for GLP-1 (antibody code no. 20 89390). Insulin and C-peptide concentrations were measured using commercial ELISA kits (code No K6219 and K6218 respectively, Dako @, Copenhagen, Denmark). Detection limits of the assays were 3 pmol/l for insulin and 17 pmol/l for C-peptide. Intra- and interassay 25 coefficients of variation were 4-10 % at 39-1240 pmol/l for insulin and 3-6 % at 380 -2700 pmol/l for C-peptide. The crossreactivity with intact and split proinsulins in the C-peptide assay was 63-87 %. HbA_{1c} was measured by ion-exchange HPLC (at Steno Diabetes Hospital, Gentofte, 30 Denmark) with an interassay coefficient of variation of 0.15 % in the range of 4.7-11.3 % (normal range: 4.1-6.4 %). FFAs were measured using an enzymatic colorimetric assay (Wako, WA, USA).

G. Calculations

[0080] For gastric emptying calculations, regions of interest for integration of radioactivity were delineated manually around the stomach on each image. Counts were corrected for physical decay, and geometric means of anterior and posterior counts were used for attenuation correction. The area under the normalized time-activity curve was used to characterize the gastric emptying rate. Insulin sensitivity was expressed as the amount of glucose infused during the last 30 minutes of the hyperinsulinemic euglycemic clamp and was presented as mg glucose/kg lean body mass/min.

15 H. Statistics

[0081] All data were presented as the mean \pm SEM. For comparisons within the groups, paired t test and repeated measures ANOVA with post hoc Bonferroni correction for normally distributed data, and Wilcoxon's matched pairs and Friedman tests with post hoc Dunn correction for non-parametric analysis are used. For comparisons between the groups (Δ values in the GLP-1 group versus Δ values in the saline group) expressing the real treatment effect, unpaired t test or one-way ANOVA for parametric analyses and Mann-Whitney or Kruskal-Wallis for non-parametric analyses was used.

2. Plasma levels of GLP-1

[0082] Plasma levels of GLP-1 in patients who had been administered saline remained unaltered at 10.8 ± 2.4 (week 0) versus 10.5 ± 2.3 (week 1) versus 9.4 ± 2.1 (week 6) pmol/l, NS. For patients receiving GLP-1, GLP-1

increased from 19.0 ± 9.7 (week 0) to 196.8 ± 27.9 (week 1) and 253.7 ± 46.1 (week 6), $P < 0.0001$ in patients treated with GLP-1. Δ values (changes in patients receiving GLP-1 versus changes in controls): $P < 0.0001$.

5

3. Plasma levels of glucose, insulin, C-peptide, glucagon and FFA

[0083] 8-h plasma glucose profiles are provided in Figure 1. For patients receiving saline, plasma glucose levels remained unchanged over the 6 weeks (Figure 1B). In patients receiving GLP-1, fasting and 8-h mean plasma glucose levels decreased by 3.4 and 4.9 mmol/l (week 1), respectively, and by 4.3 and 5.5 mmol/l (week 6), respectively, $P < 0.001$ Δ values: $P < 0.0001$ (Figure 2).

10
15 Post-prandial glucose excursions (expressed as incremental AUCs 0-3 h) decreased, $P < 0.001$, Δ values: $P < 0.0001$ as did post-prandial peak values, $P = 0.003$, Δ values: $P < 0.0001$.

[0084] In the group of patients that were administered saline, 8-h profiles of insulin, C-peptide, glucagon and FFA levels remained unaltered during the 6 weeks.

[0085] For patients receiving GLP-1, fasting insulin levels increased from 65.8 ± 12.4 (week 0) to 76.2 ± 13.3 (week 1), and to 97.9 ± 21.7 (week 6) pmol/l, $P = 0.018$ (week 0 versus week 6), Δ values: $P = 0.04$; fasting C-peptide levels increased from 955.4 ± 130.5 (week 0) to 1077.8 ± 144.3 (week 1) and to 1136.3 ± 159.4 (week 6) pmol/l, $P = 0.045$ (week 0 versus week 6), Δ values: $P = 0.017$; and fasting glucagon levels decreased from
25
30 11.6 ± 1.5 (week 0) to 9.0 ± 0.8 (week 1) and 9.4 ± 0.7 (week

6), $P=0.015$ (week 0 versus 1), Δ values: NS. 8-h mean levels, however, remained unaltered.

[0086] Fasting levels of FFA in patients that were administered GLP-1 decreased from 0.9 ± 0.08 (week 0) to 0.77 ± 0.09 (week 1) and to 0.63 ± 0.04 (week 6) mmol/l, representing a 30 % reduction, $P=0.0005$ (week 0 versus week 6); Δ values: $P=0.0014$. 8-h mean levels decreased from 0.64 ± 0.08 (week 0) to 0.55 ± 0.06 (week 1) and to 0.49 ± 0.06 (week 6) mmol/l, representing a 23% reduction, $P=0.01$, (week 0 versus week 6) Δ values: $P=0.04$.

4. Levels of HbA_{1c} and fructosamine

[0087] In patients treated with saline, HbA_{1c} was 8.9 ± 0.4 (week 0) versus 9.1 ± 0.5 (week 6) percent, NS. Fructosamine levels in saline treated patients increased from 348.7 ± 19.6 (week 0) to 381.0 ± 27.2 (week 3) and 384.6 ± 23.7 (week 6) $\mu\text{mol/l}$, $P=0.0004$.

[0088] In patients treated with GLP-1, HbA_{1c} decreased from 9.2 ± 0.6 (week 0) to 7.9 ± 0.5 (week 6) per cent, $P=0.003$. Δ values: $P=0.001$. Fructosamine levels in the GLP-1 treated patients decreased from 349.0 ± 23.1 (week 0) to 301.2 ± 15.3 (week 3) and 282.1 ± 16.1 (week 6) $\mu\text{mol/l}$, $P=0.0002$. Δ values: $P<0.0001$. The decline in HbA_{1c} would probably have been more pronounced if the GLP-1 treatment had lasted longer than 6 weeks, since it takes more than six weeks to reach new steady state levels of HbA_{1c}.

5. Gastric emptying

[0089] For patients receiving saline, gastric emptying remained unaltered. For patients receiving GLP-1, gastric emptying was significantly inhibited, thus AUC

values increased from 146.3 ± 16.5 (week 0) to 188.8 ± 18.9 (week 1) and to 189.1 ± 7.9 (week 6) counts per minute * hour, $P=0.014$. Δ values: NS.

5 **6. Insulin sensitivity**

[0090] Mean insulin levels for the last 120 minutes of the clamp were 415.8 ± 19.2 (GLP-1, week 0) and 458.7 ± 25.8 (GLP-1, week 6) compared to 399.2 ± 25.1 (saline, week 0) and 405.9 ± 40.6 (saline, week 6) pmol/l, NS (Figure 3).

10 Mean C-peptide levels for the last 120 minutes of the clamp were 269.9 ± 33.5 (GLP-1, week 0) versus 516.8 ± 84 (GLP-1, week 6) and 275.1 ± 25.5 (saline, week 0) versus 299.2 ± 48.2 (saline, week 6) pmol/l, $P=0.006$ ((GLP-1, week 6) significantly higher than the other levels).

15 [0091] Plasma glucose levels for the last 30 minutes of the clamp were 5.0 ± 0.1 (GLP-1, week 0) versus 4.9 ± 0.09 (GLP-1 week 6) and 4.8 ± 0.06 (saline, week 0) versus 4.9 ± 0.09 (saline, week 6) mmol/l, NS (Figure 4).

[0092] For saline treated patients insulin sensitivity (glucose utilisation) remained unaltered. For patients receiving GLP-1 insulin sensitivity (glucose utilization) increased by 84%, $P=0.003$, Δ values: $P=0.004$ as shown in Figure 5.

25 **7. β -cell function**

[0093] Figure 6 shows C-peptide levels during the hyperglycemic clamp. In the control group incremental AUC C-peptide levels remained unaltered. For patients receiving GLP-1, incremental AUC (0-90 minutes) C-peptide levels increased, $P=0.0013$, Δ values: $P=0.0002$. First phase responses to glucose (incremental AUC 0-10) increased, $P=0.018$ (week 0 versus 6), Δ values: $P=0.006$

(Δ week 0-6 GLP-1 versus saline). Week 0 versus week 1, $P=0.027$, Δ values: $P=0.044$. Second phase response to glucose (incremental AUC 10-45) increased, $P=0.0008$, Δ values: $P<0.0001$. Incremental maximal secretory capacity (peak levels after arginine infusion) increased, $P<0.0001$, Δ values: $P<0.0001$.

8. Body weight and body composition

[0094] For patients receiving saline, body weight decreased insignificantly from 93.3 ± 3.8 (week 0) to 92.6 ± 3.7 (week 6) kilograms. Fat mass in per cent was unaltered at $34.4\pm 2.2\%$ (week 0) versus $34\pm 2.7\%$ (week 6)

[0095] During GLP-1 treatment body weight decreased from 104.8 ± 5.9 (week 0) to 102.9 ± 5.9 (week 6) kilograms, $P=0.02$. Δ values: NS ($P=0.13$). Total fat mass decreased from 40.1 ± 4.3 (week 0) to 38.9 ± 3.9 (week 6) kilograms, NS. Total lean body mass was 61.7 ± 3.7 (week 0) versus 61.1 ± 3.6 (week 0) kilograms, NS. Thus, percent fat mass was unaltered at 37.7 ± 2.9 (week 0) versus 37.2 ± 2.6 (week 6) %, NS.

9. Appetite and other effects

[0096] In the saline group, sensations of hunger, satiety, fullness and prospective food intake. For patients receiving GLP-1, sensation of hunger decreased from 247 ± 33.8 (week 0) to 182 ± 44 (week 1) and 182 ± 23.8 (week 6) mm * hour, $P=0.02$. Δ values: $P=0.005$ (week 0 versus week 1). For patients receiving GLP-1, sensation of satiety increased from 347.4 ± 29.7 (week 0) to 419.9 ± 38.5 (week 1) and 411.5 ± 29 (week 6) mm * hour, $P=0.03$ (week 0 versus 1). Δ values: NS. For patients receiving GLP-1, sensation of fullness increased from

308.2±24.5 (week 0) to 420.6±43.3 (week 1) and 398.5±35.2 (week 6) mm * hour, P=0.008. Δvalues: P=0.05 (week 0 versus week 1).

[0097] For patients receiving GLP-1, sensation of prospective food intake decreased from 312.9±32 (week 0) to 227±43.6 (week 1) and 217±24.9 (week 6) mm * hour, P=0.0073. Δvalues: P=0.04 (week 0 versus week 1).

10. Effect of GLP-1 on reversing severe left ventricular dysfunction in dogs with pacing-induced dilated cardiomyopathy.

[0098] Dilated cardiomyopathy was induced in conscious dogs by rapid ventricular pacing at 210 beats per minute over 28 days. As shown in Figure 7, the pacing was associated with a significant rise in serum glucose (90±2 to 115±4 mg%), insulin (27±2 to 75±8 pmol/l), free fatty acids 269±43 to 702±50 μmol/l) and norepinephrine (83±8 to 320±48 pg/ml). All increases were statistically significant. The combination of elevated glucose in the presence of increased levels of insulin is pathognomonic of insulin resistance. Figure 8 demonstrates the changes in uptake of free fatty acids versus glucose into heart muscle that occur with cardiomyopathy measured at 28 days of pacing and following 48 hours of treatment with placebo or GLP-1 (5 ng/kg/min). Prior to induction of cardiomyopathy, the ratio of uptake of free fatty acids (FFA) to glucose was nearly 1.0, indicating approximately equal uptake. After 28 days of pacing, the ratio is approximately 1.5, indicating that FFA are preferred over glucose. Following treatment with GLP-1, the ratio is reversed (0.5), indicating that GLP-1 preferentially enhances the uptake of glucose over FFA in the cardiomyopathy heart. Figure 9 demonstrates the

physiological consequences of GLP1 in pacing-induced cardiomyopathy. Compared to placebo, GLP-1 increases stroke volume ($p < 0.001$), left ventricular (LV) systolic pressure ($p < 0.001$) and LV dP/dT ($p < 0.001$) and decreases
5 heart rate ($p < 0.005$). These changes demonstrate that GLP-1 reverses an underlying metabolic myopathy of dilated cardiomyopathy that is associated with insulin resistance and severe left ventricular dysfunction.

Claims

We Claim:

1. A method of treating a subject suffering from an insulin resistance-associated condition comprising a step of administering a therapeutically effective amount of GLP-1.
2. The method according to claim 1, wherein the insulin resistance-associated condition is selected from the group consisting of type-2 pre-diabetes, ASCD, drug-induced insulin resistance, congestive heart failure, diminished exercise capacity of skeletal muscle, and left ventricular dysfunction with cardiac metabolic myopathy or diminished exercise capacity of skeletal muscle; with the proviso that said congestive heart failure is not associated with toxic hypervolemia.
3. The method according to claim 1, wherein the insulin resistance-associated condition is type-2 pre-diabetes.
4. The method according to claim 1, wherein the insulin resistance-associated condition is ASCD.
5. The method according to claim 1, wherein the insulin resistance-associated condition is drug-induced insulin resistance muscle.
6. The method according to claim 1, wherein the insulin resistance-associated condition is congestive heart failure, with the proviso that said congestive heart failure is not associated with toxic hypervolemia.

7. The method according to claim 1, wherein the insulin resistance-associated condition is left ventricular dysfunction with cardiac metabolic myopathy or diminished exercise capacity of skeletal muscle.

8. The method according to claim 2 or 5, wherein the drug-induced insulin resistance is glucocorticoid-induced insulin resistance or growth hormone-induced insulin resistance.

9. The method according to any one of claims 1 to 7, wherein the subject is a human.

10. The method according to any one of claims 1 to 7, wherein the GLP-1 molecule is selected from the group consisting of GLP-1(7-36)NH₂, GLP-1(9-36)NH₂, GLP-4(7-37), and exendin-4.

11. The method according to claim 10, wherein the GLP-1 molecule is selected from the group consisting of GLP-1(7-36)NH₂ and GLP-1(9-36)NH₂.

12. The method according to claim 10, wherein the GLP-1 molecule is GLP-1(7-36)NH₂.

13. The method according to claim 10, wherein the GLP-1 molecule is GLP-1(9-36)NH₂.

14. The method according to any one of the claims 1-7, wherein the GLP-1 molecule is administered for a period of time selected from the group consisting of at least 6 weeks, at least 8 weeks, at least 10 weeks, at least 12 weeks and at least 14 weeks.

15. The method according to claim 14, wherein the GLP-1 is administered by an infusion pump or by subcutaneous injection of a slow release formulation.

1/9

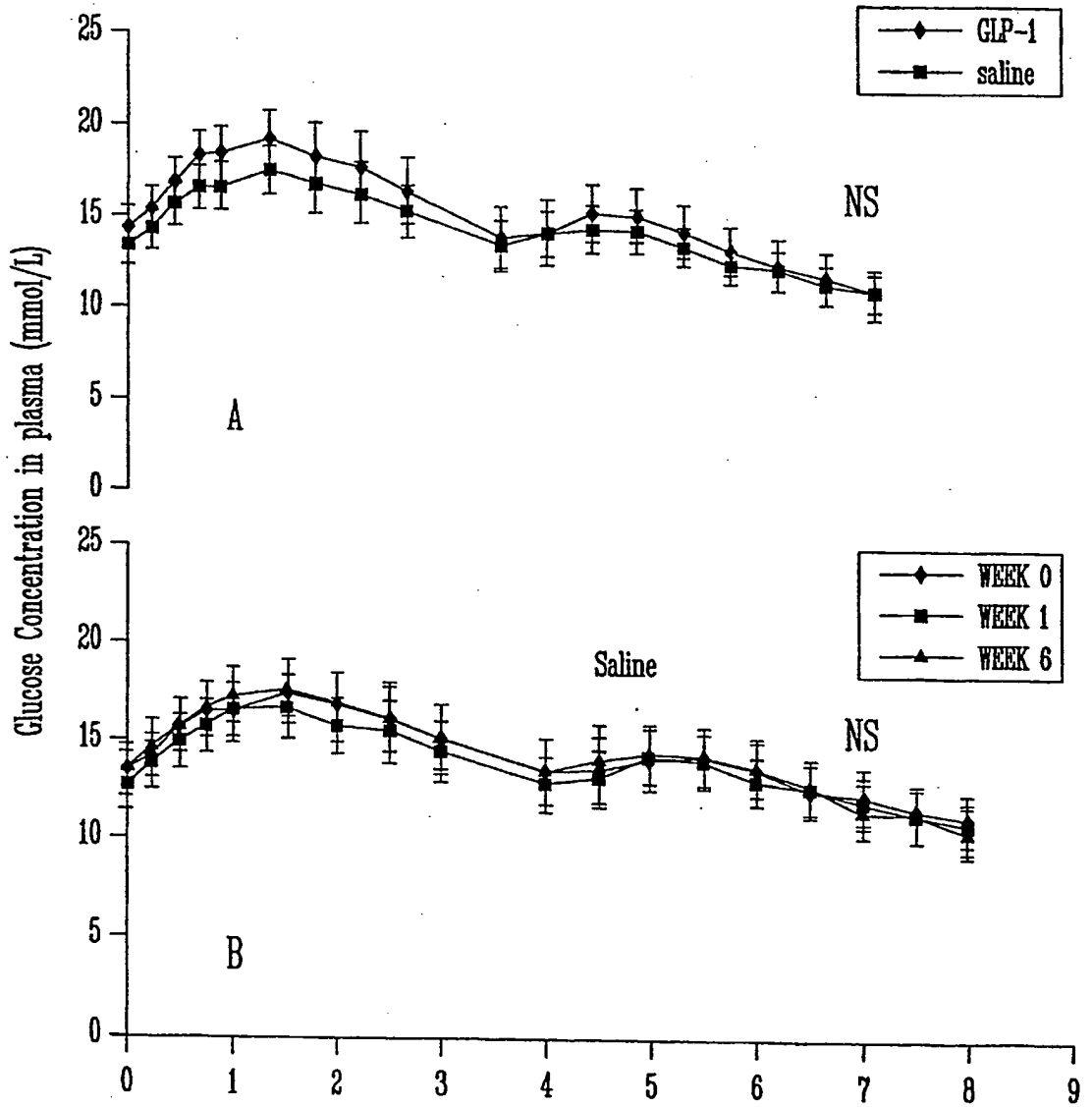


Fig. 1

2/9

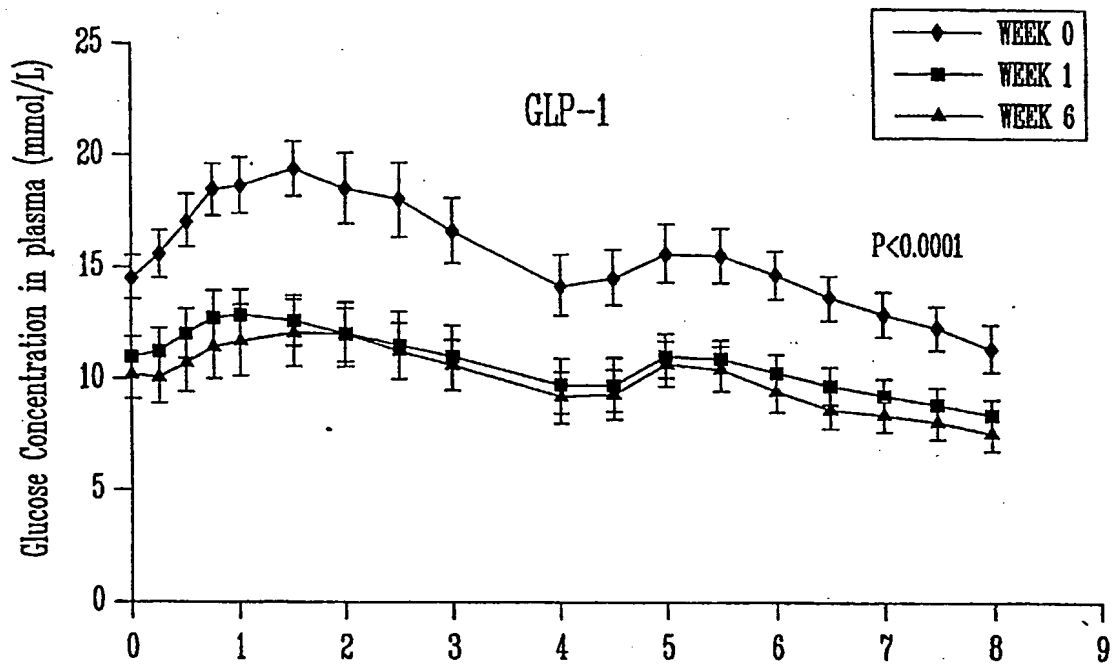


Fig. 2

3/9

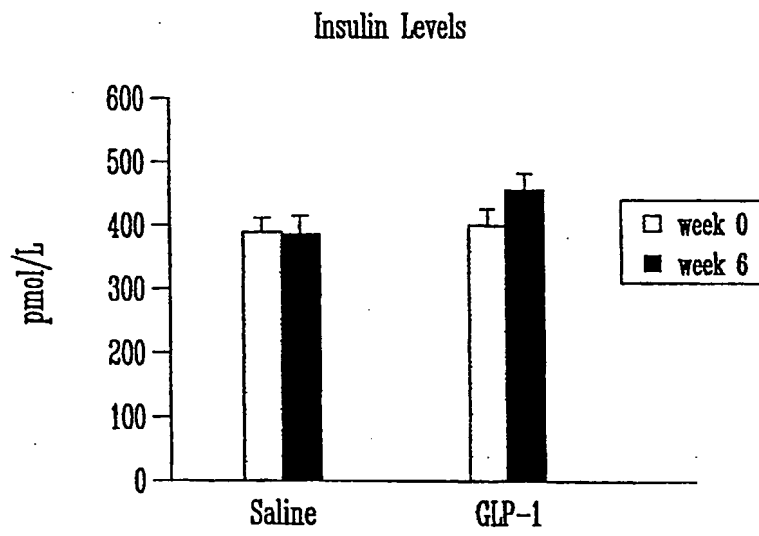


Fig. 3

4/9

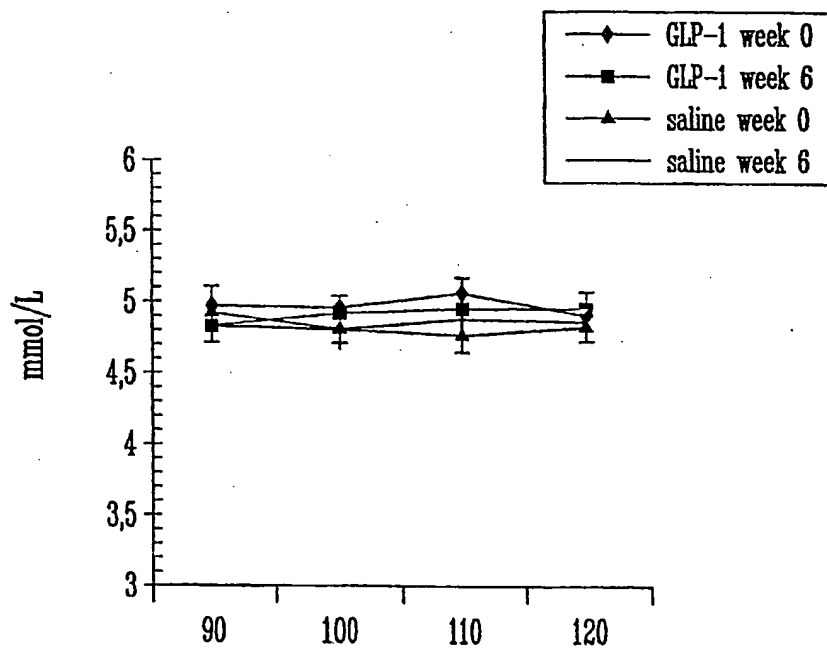


Fig. 4

5/9

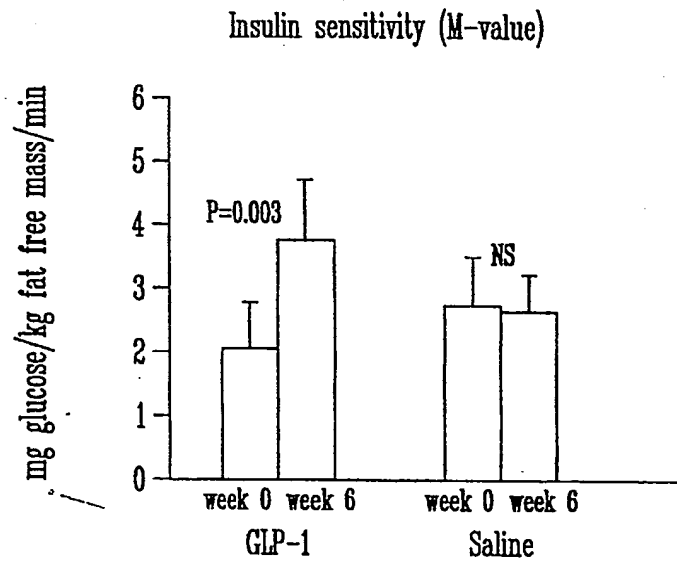


Fig. 5

5/9

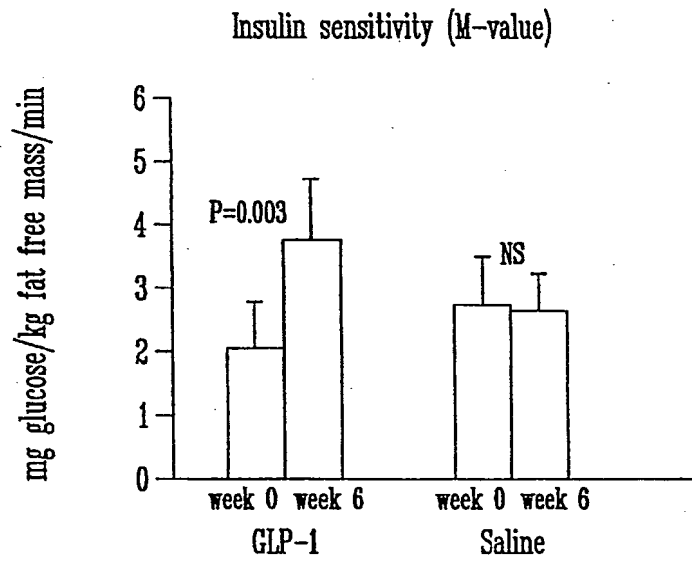


Fig. 5

6/9

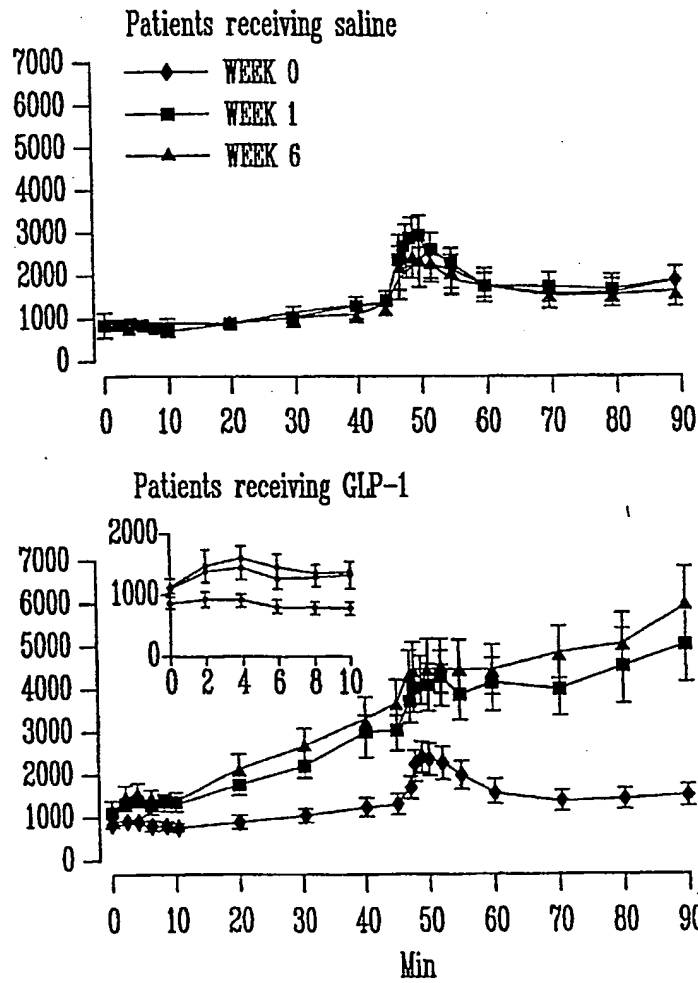


Fig. 6

7/9

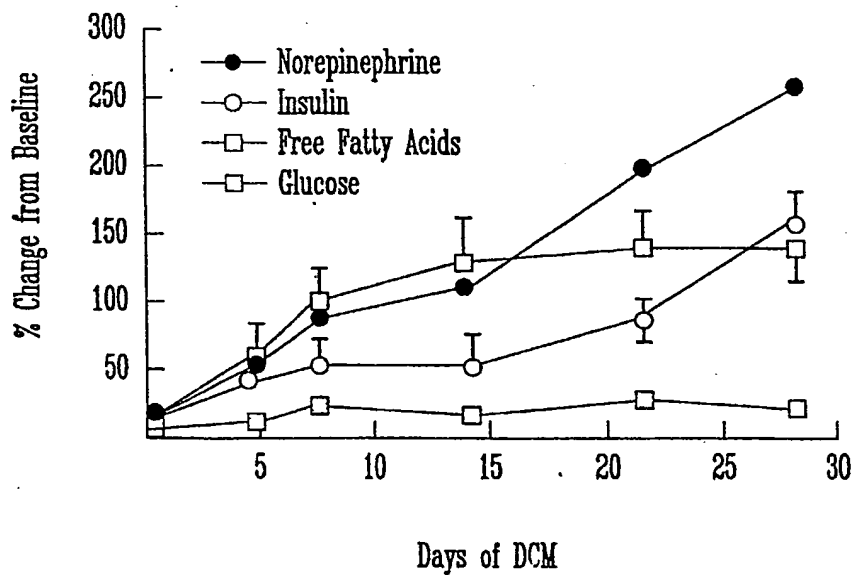


Fig. 7

8/9

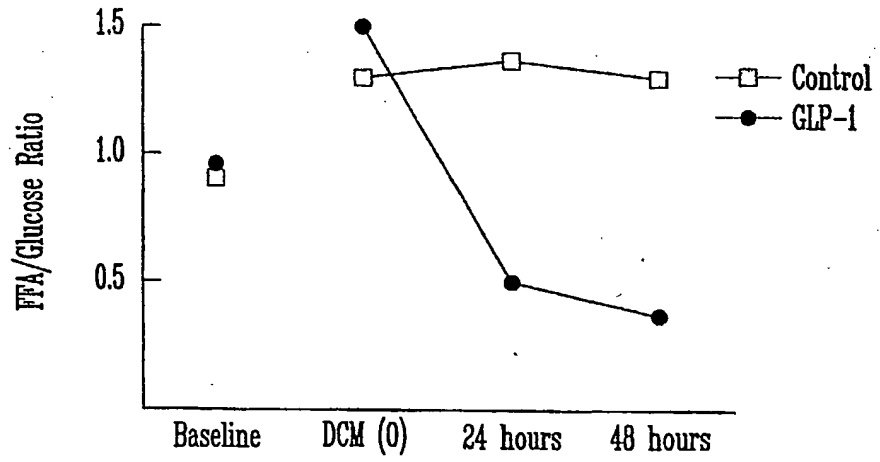


Fig. 8

9/9

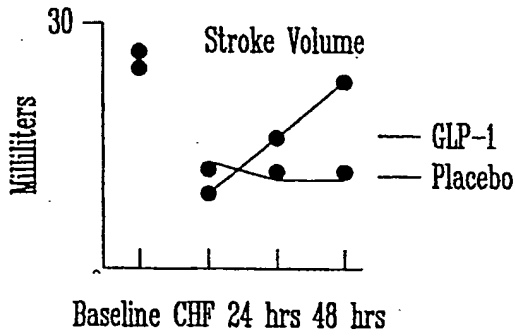


Fig. 9A

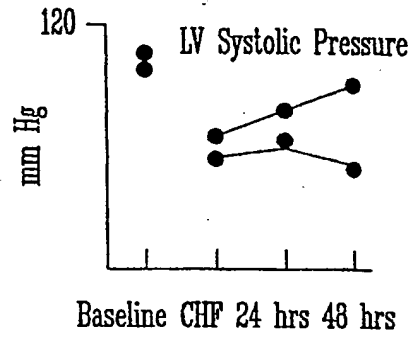


Fig. 9B

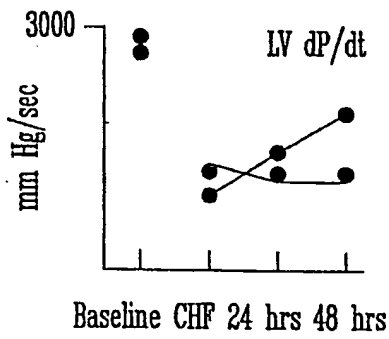


Fig. 9C

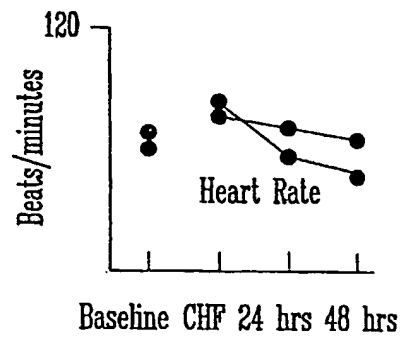


Fig. 9D

RGN-3 PCTIN THE UNITED STATES PATENT OFFICE AS RECEIVING OFFICE
UNDER THE PATENT COOPERATION TREATY

Applicant : Restoragen, Inc.
Application No.: Not Yet Assigned
Filed : Concurrently Herewith
For : METHODS AND COMPOSITIONS FOR
TREATING CONDITIONS ASSOCIATED WITH
INSULIN RESISTANCE

New York, New York
April 24, 2002

Hon. Commissioner for Patents
P. O. BOX 2327
Arlington, VA 22202

Attention: BOX PCT
RO/US

STATEMENT ACCOMPANYING SEQUENCE LISTING

In accordance with PCT Rule 13^{ter}.1(b), the undersigned hereby states that the Sequence Listing submitted concurrently herewith does not include matter which goes beyond the content of the international application as filed and that the information recorded in computer readable form submitted concurrently herewith is identical to the written Sequence Listing.

SEQUENCE LISTING

<110> Restoragen, Inc.

<120> METHODS AND COMPOSITIONS FOR TREATING CONDITIONS ASSOCIATED WITH INSULIN RESISTANCE

<130> RGN-3

<160> 13

<170> PatentIn version 3.1

<210> 1

<211> 37

<212> PRT

<213> mammalian

<400> 1 .

His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val
 1 5 10 15

Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu
 20 25 30

Val Lys Gly Arg Gly
 35

<210> 2

<211> 36

<212> PRT

<213> mammalian

<400> 2

His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val
 1 5 10 15

Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu
 20 25 30

Val Lys Gly Arg
 35

<210> 3

<211> 31

<212> PRT

<213> mammalian

<400> 3

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 20 25 30

<210> 4

<211> 30

<212> PRT

<213> mammalian

<400> 4

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
 20 25 30

<210> 5

<211> 29

<212> PRT

<213> mammalian

<400> 5

Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala
 1 5 10 15

Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly

20

25

<210> 6

<211> 28

<212> PRT

<213> mammalian

<400> 6

Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala
 1 5 10 15

Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
 20 25

<210> 7

<211> 39

<212> PRT

<213> Gila Monster venom

<400> 7

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
 1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
 20 25 30

Ser Gly Ala Pro Pro Pro Ser
 35

<210> 8

<211> 31

<212> PRT

<213> Gila Monster venom

<400> 8

Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu
 1 5 10 15

Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser
 20 25 30

<210> 9

<211> 39

<212> PRT

<213> Gila Monster venom

<400> 9

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
 1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
 20 25 30

Ser Gly Ala Pro Pro Pro Ser
 35

<210> 10

<211> 38

<212> PRT

<213> Gila Monster venom

<400> 10

His Ser Asp Ala Thr Phe Thr Ala Glu Tyr Ser Lys Leu Leu Ala Lys
 1 5 10 15

Leu Ala Leu Gln Lys Tyr Leu Glu Ser Ile Leu Gly Ser Ser Thr Ser
 20 25 30

Pro Arg Pro Pro Ser Ser
 35

<210> 11

<211> 37

<212> PRT

<213> Gila Monster venom

<400> 11

His Ser Asp Ala Thr Phe Thr Ala Glu Tyr Ser Lys Leu Leu Ala Lys
 1 5 10 15

Leu Ala Leu Gln Lys Tyr Leu Glu Ser Ile Leu Gly Ser Ser Thr Ser
 20 25 30

Pro Arg Pro Pro Ser
 35

<210> 12

<211> 35

<212> PRT

<213> Gila Monster venom

<400> 12

His Ser Asp Ala Ile Phe Thr Glu Glu Tyr Ser Lys Leu Leu Ala Lys
 1 5 10 15

Leu Ala Leu Gln Lys Tyr Leu Ala Ser Ile Leu Gly Ser Arg Thr Ser
 20 25 30

Pro Pro Pro
 35

<210> 13

<211> 35

<212> PRT

<213> Gila Monster venom

<400> 13

His Ser Asp Ala Ile Phe Thr Gln Gln Tyr Ser Lys Leu Leu Ala Lys
 1 5 10 15

Leu Ala Leu Gln Lys Tyr Leu Ala Ser Ile Leu Gly Ser Arg Thr Ser
 20 25 30

Pro Pro Pro
 35

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/13088

A. CLASSIFICATION OF SUBJECT MATTER		
IPC(7) : A61K 38/26 US CL : 530/308; 514/12		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) U.S. : 530/308; 514/12		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,006,753 A (EFENDIC) 28 December 1999 (28.12.1999), column 4, lines 8-67.	1, 9-12
Y		1-15
Y	US 6,204,281 B1 (WEBB et al.) 20 March 2001 (20.03.2001), column 3, lines 11-41	1, 2, 6, 9-15
Y	US 5,128,320 A (HAHN et al.) 07 July 1992 (07.07.1992), column 11, lines 31-44.	1, 2, 5, 8, 9-15
Y	US 6,207,690 B1 (URBAN et al.) 27 March 2001 (27.03.2001), column 1, lines 23-43.	1-3, 9-15
Y	US 6,093,697 A (LARNER et al.) 25 July 2000 (25.07.2000), column 1, lines 50-65.	1, 2, 4, 7, 9-15
Y	US 5,496,831 A (ALEXANDER-BRIDGES et al.) 05 March 1996 (05.03.1996), column 6, lines 18-34.	1, 2, 6, 9-15
Y	US 5,798,102 A (MCMICHAEL et al.) 25 August 1998 (25.08.1998), column 5, lines 29-40.	1, 2, 6, 7, 9-15
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search	Date of mailing of the international search report	
02 July 2002 (02.07.2002)	18 SEP 2002	
Name and mailing address of the ISA/US	Authorized officer	
Commissioner of Patents and Trademarks Box FCT Washington, D.C. 20231	DANIEL S. ROMEO	
Facsimile No. (703)305-3230	Telephone No. 703 308-0196	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/13088

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YOUNG et al. Glucose-lowering and insulin-sensitizing actions of exendin-4: Studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (<i>Macaca mulatta</i>). <i>Diabetes</i> . May 1999, Vol. 48 No. 5, pages 1026-1034, especially page 1033, left column, last full paragraph.	1-15
Y	GREIG et al. Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. <i>Diabetologia</i> . January 1999, Vol. 42 No. 1, pages 45-50, especially pages 45-46.	1-15
Y	KELLEY, D.B. Overview: What is insulin resistance? <i>Nutrition Reviews</i> . March 2000, Vol. 58, No. 3, Pt. 2, pages S2-S3, especially pages S2-S3, left and right columns.	1-15
Y	US 5,574,008 A (JOHNSON et al.) 12 November 1996 (12.11.1996), column 2, lines 20-32, paragraph bridging columns 8-9.	1-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/13088

Continuation of B. FIELDS SEARCHED Item 3:
APS, MEDLINE
search terms: glucagon-like peptide, insulin resistance